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KAMINISTIQUIA RIVER
MISA PILOT SITE STUDY:
ALGAL ASSAY

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KAMINISTIQUIA RIVER

MISA PILOT SITE STUDY

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MISA PILOT SITE - KAMINISTIQUIA RIVER (THUNDER BAY) - ALGAL ASSAY

INTRODUCTION

To evaluate a possible toxic effect of the Canadian Pacific Forest Products Ltd., effluent on phytoplankton growth and community structure, a semicontinuous assay was conducted in the laboratory from August 21 to September 3, 1986. The previous attempt of incubating algae in-situ, in August 1985 along the effluent gradient in Kaministiquia River using the "caged" phytoplankton method (Nicholls and Heintsch 1988 unpublished), was not successful because there was an extensive deposition of filamentous bacteria of the "sewage fungus" community on the bottles, preventing the nutrient and light penetration inside the bottles.

In our semicontinuous assay experiment effluent evaluation was achieved by exposing a portion of a natural algal community from the Kaministiquia River to a range of effluent concentrations and by evaluating toxicity not only as a biomass yield, but as a taxonomic composition change.

The majority of algal assays use a single species' response as a toxicity measure. This may not represent the natural community reaction to the toxicants, especially if a complex waste, such as the pulp and paper mill effluent is tested. Algal community response to toxicants is a resultant of individual responses of community members. In natural communities interspecific competition modifies the toxic effect of pollutants, e.g. the less sensitive fast growing species absorb the toxicants and allow the survival of more sensitive but slow growing species. Toxicants may also affect the population dynamics resulting in dominance of the resistant species (Walsh and Merril 1984). Changes in algal community structure may have effects on herbivore population, subsequently affecting the structure and density of populations at higher levels in the food chain.

We chose a semicontinuous assay for this evaluation. This method facilitated not only a semicontinuous nutrient supply and dilution of algal metabolites, but also an addition of effluent which compensated toxicity losses through an absorption by algae.

MATERIALS AND METHODS

Inoculum and Effluent Collection

The inoculum was collected as a photic zone composite sample at the control station located upstream from the paper mill (Hwy. 61 bridge) on August 20. The sample was shipped to the laboratory in a cooler within four hours and then stored in the dark at 5°C until August 21 (1 day) when it was concentrated by filtration through 0.45 milipore filter. Ten mL of this concentrated sample containing 1.4 $\mu m^3 \cdot mL^{-1}$ of algae were inoculated into each flask immediately after the filtration. The cells were not predepleted of their internal phosphorus reserves prior to the assay, since they came from the source (upper Kaministiquia River) with relatively low phosphorus concentration (0.012 $mg \cdot L^{-1}$). The inoculum obtained as a sample of a natural algal community was not bacteria free.

A subsample of a 24 h composite "dirty outfall" GLA effluent collected on August 13-14, 1986, and the Kaministiquia River water from a control station (Hwy. 61 bridge) were used in the assay. The effluent and the control station water were shipped unrefrigerated in 1 litre glass bottles, and arrived in the laboratory within 5 days (August 19). Both were filtered through a 0.45 milipore membrane on August 19 and 20 and stored at 5°C until August 21, the beginning of the assay.

Nutrient Spike

Muskoka medium (Wehr *et al*. 1985) was used as a nutrient spike to eliminate the stimulatory effect of high nutrient concentration in the effluent. The "f/2" vitamin mix (Nichols 1973) containing thiamine 0.2 mg/L, biotin 0.001 mg/L and B 0.001 mg/L was also added. The medium was modified by an addition of 19.868 mg/L of $Na_2SiO_3 \cdot 9\ H_2O$ to achieve 5 mg SiO_3/L to ensure diatom growth and the addition of 132.00 mg/L of $(NH_4)_2SO_4$ to decrease the N/P ratio. EDTA was omitted from the solution so that the presence of introduced organic chelates was minimized.

Bioassay Design

The bioassay involved 5 treatments with different concentrations of effluent, diluted with the Kaministiquia River water enriched with a growth medium, and a control treatment consisting of the enriched river water (Table 1).

Each treatment consisted of a triplicate incubation of 200 mL of culture in 500 mL Pyrex borosilicate glass Ehrlenmayer flasks. The flasks were agitated by a shaker, and incubated in a growth chamber under 12:12 hour light - dark cycle. White fluorescent light was provided at the intensity of: 300 microeinsteins m^{-2} sec^{-1} for 2 hours, 480 microeinsteins m^{-2} sec^{-1} for 8 hours, 300 microeinsteins m^{-2} sec^{-1} for 2 hours. The varied light intensity was set up to imitate the varied light intensity in a natural environment. The temperature was set at 15°C but it oscillated daily between 10° - 18°C.

Cultures were manually diluted every 48 h by removing 100 mL of culture suspension and replacing it with an equal volume of medium starting on the fifth day of the incubation (the estimated end of a lag phase). There were six transfers.

Table 1: The Bioassay Design

	Filtered River Water + Inoculum mL	Filtered Effluent mL	Muskoka Medium mL
Control	180	0	20
70% effluent	40	140	20
40% effluent	100	80	20
10% effluent	160	20	20
2% effluent	176	4	20
0.5% effluent	179	1	20

A 10 mL aliquot of the culture was sampled from each flask at the time of each transfer, and at the end of the assay. The samples were preserved with Lugol's iodine solution for algal biomass analysis. The biomass was estimated as a biovolume expressed as $\mu m^3 \cdot mL^{-1}$, by measuring the algae in a Utermöhl counting chamber using a Whipple grid and an inverted microscope at 600X magnification. The growth rate μ was estimated using the formula: $\mu = \log (x_2/x_1)/t_2 - t_1$ (Miller et al. 1978), where x = algal biovolume, and t = incubation time.

A nonparametric analysis of variance using Kruskall-Wallis tests followed by Tukey-type multiple comparisons was choosen to analyze the data.

RESULTS

There was a considerable growth response (Fig. 1) to all the effluent concentrations during the first week of the assay (August 26 and August 28 transfers), expressed both as a growth rate (Fig. 2), and as a percent of the control biovolume (Fig. 3).

The algae responded to all effluent concentrations by a reduced growth rate (Fig. 2) this response was most pronounced in the 70% and 40% effluent concentrations, where the growth rates were reduced by 69% and 64% relative to the control. The growth rate reductions for the 10%, 2% and 0.5% effluent concentrations were 55%, 48% and 50% respectively. Corresponding to the reduced growth rates the algae biovolumes at all the effluent treatments were less than 50% of the control biovolume (Fig. 3) on the fifth and seventh day of the assay, despite the higher nutrient concentrations (Table 2), and were significantly different ($P > 0.05$) from the control.

The biovolume at the highest effluent concentration remained low, and comprised only 26% of the control biovolume at the end of the experi-

Fig. 1: The growth response of the portion of Kaministiquia River algal community to different effluent concentrations.

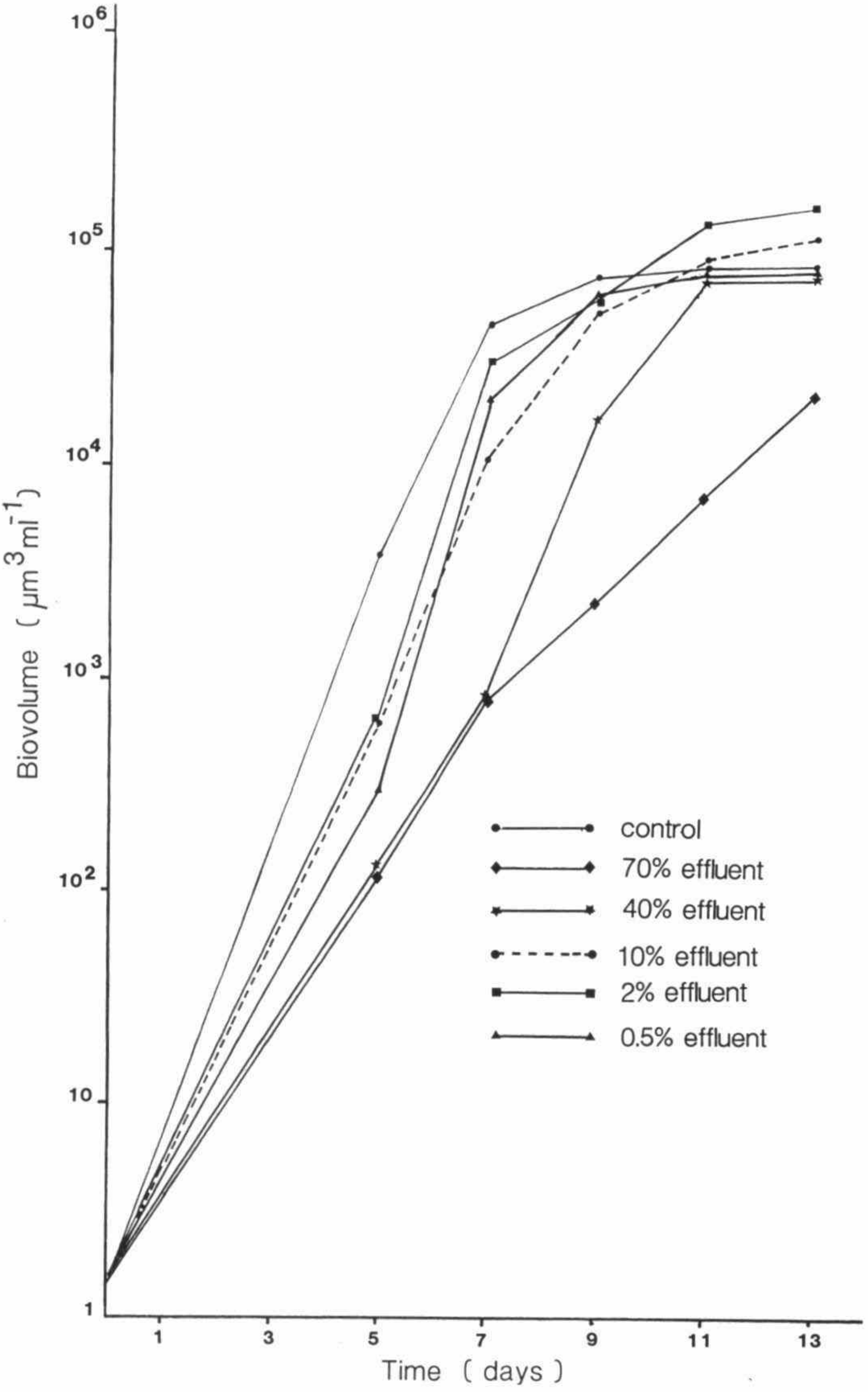


Fig. 2: Growth rate ($\mu m^3 \cdot mL^{-1} day^{-1}$) in control and the effluent concentrations of: 0.5, 2, 10, 40 and 70%

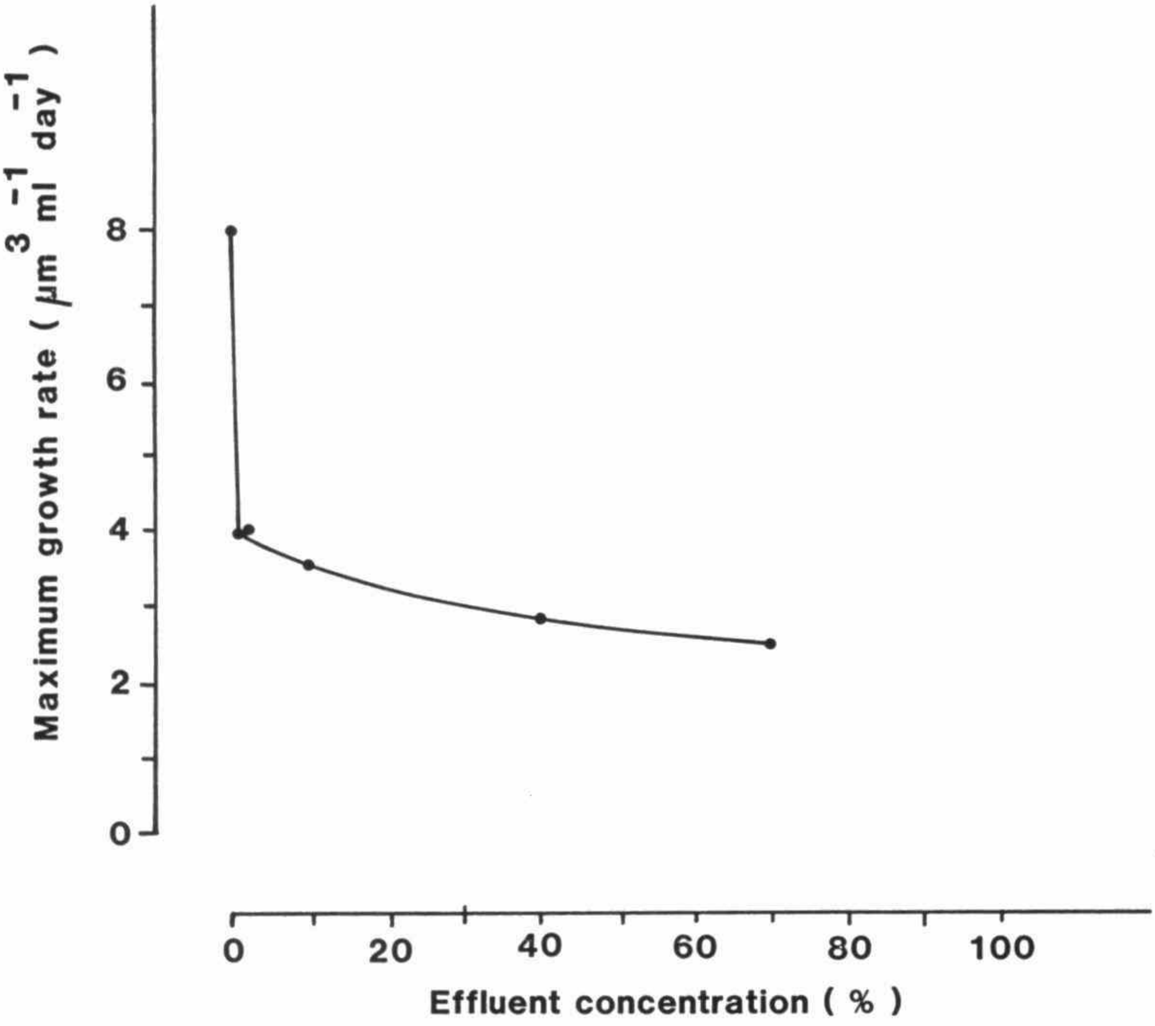


Fig. 3: Growth of algae in different effluent concentrations expressed as percent of control biovolume.

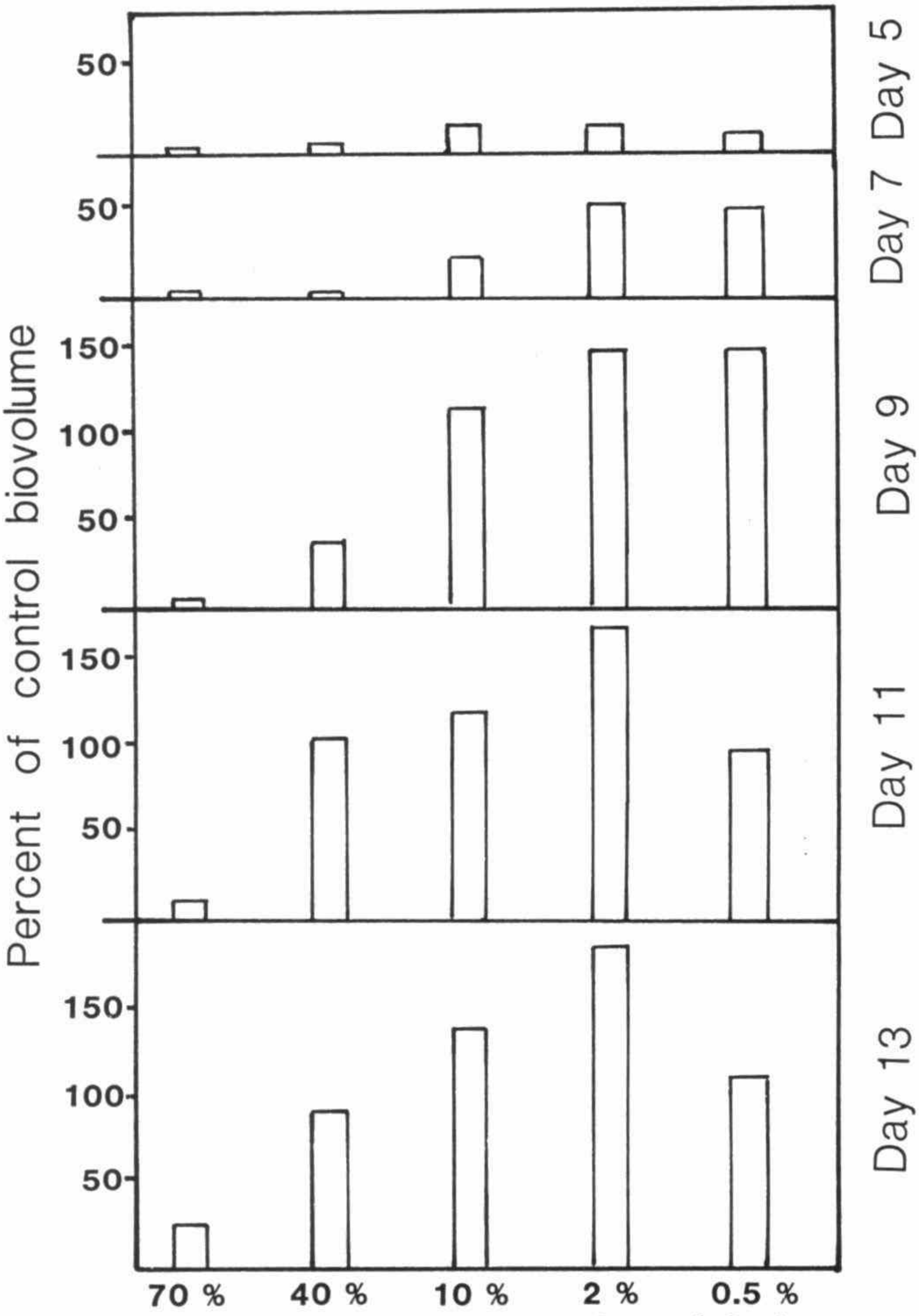


Table 2: Initial phosphorus and nitrogen concentrations (mg/L) in the growth medium.

Growth Medium	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	N:P Ratio
Control	0.315	3.032	10:1
70% Effluent	2.396	4.72	3:1
40% Effluent	1.502	4.16	3:1
10% Effluent	0.608	3.312	5:1
2% Effluent	0.370	3.124	8:1
0.5% Effluent	0.325	3.095	9:1

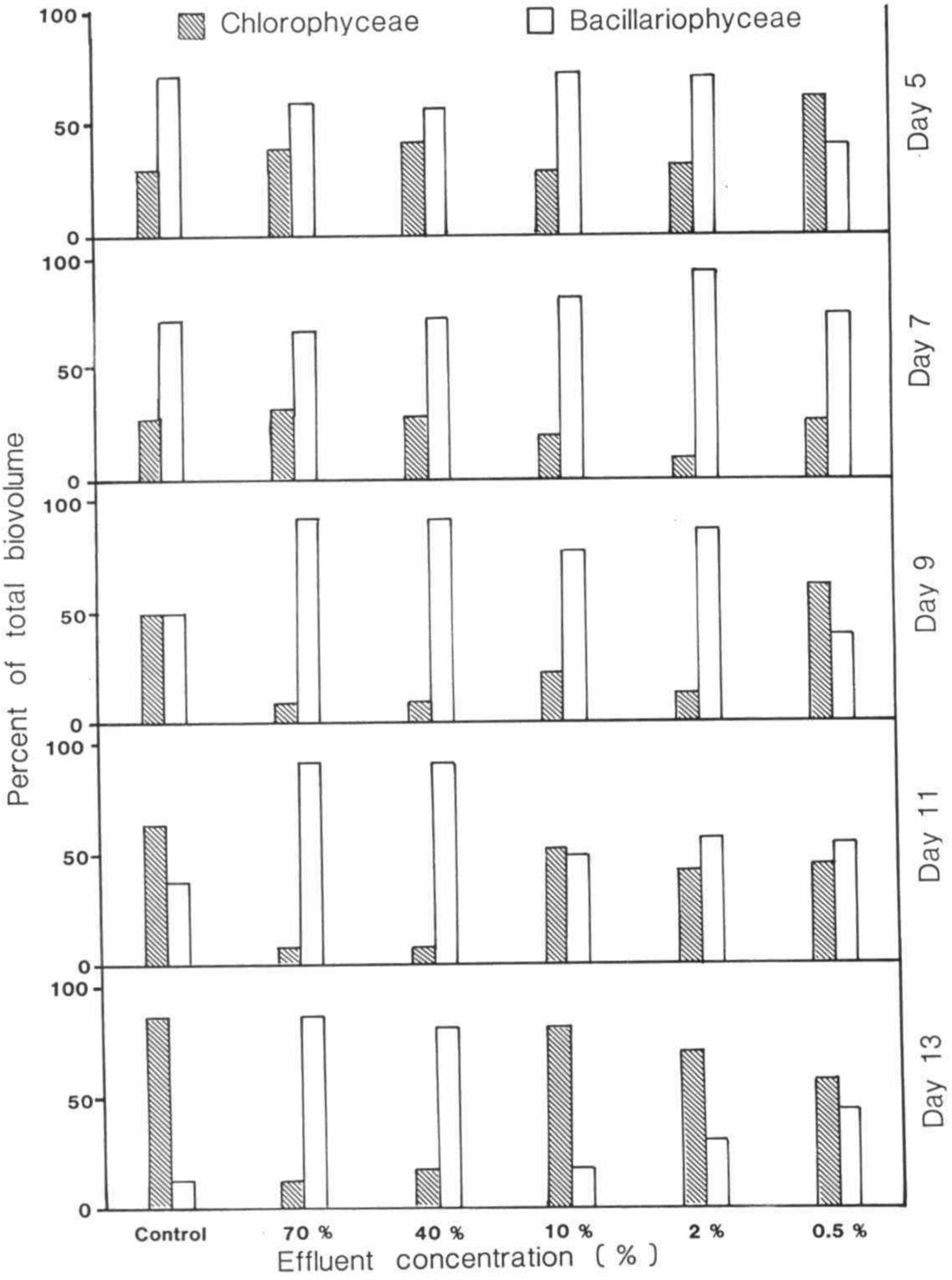
ment (Fig. 3). The biovolume in the treatment with the 40% effluent reached the control treatment level on the eleventh day. On the ninth day the inhibitory effect of the 10%, 2% and 0.5% effluent was overcome by a stimulatory effect of nutrients resulting in biovolumes greater than in the control treatment, although only the 2% effluent biovolume was significantly higher (P >0.05).

A high variability among the replicates, possibly caused by a high variability component present in natural communities; i.e. cells were not synchronized concerning their division cycle (Fogg 1965) made the use of a parametric analysis of variance impossible, due to the lack of homogeneity among variances (Zar 1984).

The algae in the inoculum were represented by 33 genera distributed among six classes, Melosira, Oocystis, Chlamydomonas, Cyclotella and Gloeocystis were the most dominant genera, Chlorophyceae and Bacillariophyceae the most, and equally dominant classes. At the beginning of the incubation many genera declined, and only the Chlorophyceae and Bacillariophyceae were found in the flasks. This reduction of algae diversity was common to all the flasks including control, and it was considered to be caused by the container effect (Fogg 1965) rather than by the toxicity of the effluent. The most dominant genera in all the treatments during the whole incubation period were : Chlamydomonas, Scenesdesmus, Monoraphidium, Nitzschia and Cyclotella.

Although the dominant genera were found in all the treatments, a shift in class dominance from Bacillariophyceae to Chlorophyceae was noted towards the end of the assay (Fig. 4). There was an initial decline of green algae (August 28 transfer) followed by a recovery leading to a dominance at the end of the incubation, with an exception of 70% and 40% effluent concentrations, where the diatoms were increasingly dominant. This trend suggests there was a bottle effect combined with the toxicity of the two highest concentrations of the effluent.

Fig. 4: Percent contribution of Chlorophyceae and Bacillariophyceae to the total biovolume during the duration of the assay.



CONCLUSIONS

1. The Canadian Pacific Forest Procuts Ltd., effluent affected the portion of the Kaministiquia River algal community both by reducing and stimulating the growth rate and biomass production, and by altering the taxonomic composition.
2. All the effluent concentrations in the 70%-0.5% range caused a significant ($\underline{P} > 0.05$) short term (5 days) growth inhibition.
3. The inhibition in the 70% effluent persisted during the whole experiment (2 weeks).
4. The inhibition in 10%, 2% and 0.5% effluent was replaced by a stimulatory effect of nutrients towards the end of the assay.
5. The two highest effluent concentrations altered the taxonomic composition by inhibiting the growth of green algae significantly ($\underline{P} > 0.05$).

The growth inhibition was an immediate short time response to the toxicity, while the taxonomic composition change was a gradual longer term process. There may have been numerous reasons for the growth recovery; the organic and inorganic components of the effluent become biologically decomposed, the algae developed the resistance to the toxicant, or the ratio of algal biomass to toxicant concentration increased to a level favourable to more rapid algal growth (Walsh and Merril, 1984). Nevertheless a dramatic initial suppression of growth indicated the effluent had a toxic effect on this portion of the Kaministiquia River algal community. It is not possible to make a definite statement as to the effect this taxonomic composition change would have on the Kaministiquia River community.

It is impossible to determine which component of the tested effluent had the most toxic effect, because the toxicity of such a complex waste is probably related to interactions among the components.

TECHNIQUE ASSESSMENT

The algal assay is a good method for the assessment of the environmental impact of effluent discharges. Algae are directly affected by effluents containing nutrients and toxic contaminants, and are most suitable for the assessment of the stimulatory and inhibitory effects of complex wastes. Research shows algae are more sensitive to complex effluents than other organisms (Walsh and Merril 1984). As primary producers, algae are a very important component of aquatic communities and should be used for the evaluation of pollution. Two algal assays were used in this study:

1. The "caged" phytoplankton, 5 day incubation in-situ at two stations along the effluent gradient, and at the control station above the paper mill.

2. The semicontinuous 14 day laboratory algal assay.

Both methods involved incubation of portions of the indigenous Kaministiquia River algal community in different effluent concentrations.

Although the "caged phytoplankton" method was found very useful at other locations for nutrient evaluation in-situ, it was unsuitable for the effluent assessment at the Kaministiquia River MISA pilot site. The relatively high water temperatures (19°C in August 1985) combined with high BOD values in the vicinity of the paper mill, promoted an excessive bacteria and fungi growth on the incubation bottles, preventing the exchange between the ambient water and the bottles.

The semicontinuous 14 days test seemed to be a good evaluation method of the Canadian Pacific Forest Products Ltd., effluent collected on August 13-14 1986. As a semicontinuous, flow-through multispecie test, it was useful in determining the effluent effect on the natural community, such as changes in taxonomic composition and rates of primary production. This test was better in imitating natural conditions than the single species static test. As algae have a short life cycle, the 14 day test provided a multigeneration study, and chronic toxicity information. There are some drawbacks in the use of this technique, such as:

1. The test provides only a qualitative information about the toxicity of the effluent as a complex waste.

2. To develop a guideline for a given site the test needs to be performed several times throughout the year, because the indigenous community of the investigated site and the effluent characteristic varies seasonally.

3. The results obtained in the laboratory may differ from the in-situ results, since the relationship between the effects of toxicants and the environmental conditions differs in-situ from that in the laboratory.

Nevertheless, the technique has a potential in setting of effluent limits and in ongoing monitoring of compliance, anticipated as no observable effect.

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KAMINISTIQUIA RIVER MISA PILOT SITE
THE TECHNIQUE ASSESSMENT QUESTIONNAIRE

A. Field Work and Laboratory Analysis

1. Several two week assays should be performed on effluents with different characteristics. Two staff per survey would be necessary.

2. The laboratory equipment necessary for conducting algal assays stored in the "Materials and Methods" chapter of the Kaministiquia River algal assay report.

3. Does not apply.

4. About 90 samples are generated during the assay. Sample analysis time would be 2.5 months approximately, plus one month for data processing and report writing.

5. Does not apply, as it is not a field study.

B. Interpretation of Results

1. One person - an algal assay specialist. The time for the interpretation - one month.

2. Software for statistical analysis, a graphics package.

3. The results are useful for the detection of acute and chronic toxicity and also for the stimulatory effect of effluents. More tests are needed to assess the reliability of the results.

4. The data interpretation problems may be caused by:

    a. Seasonal and year-to-year variability of effluent and algal community composition.

b. The high variability among replicates of each treatment which may be greater than the variability among treatments.

This high variability among the replicates is possibly caused by a high variability component present in natural communities. Also an error in experiment set-up may occur.

c. Potential masking of secondary contaminant by an acutely toxic parameter, such as chlorine.

5. the data is only valuable in assessing the effluent as a whole, and provides a qualitative information only.

6. Not advisable from QA/QC point of view.

C. Technique Utility with MISA Context

1. More testing is needed to see if the guidelines based on this technique can be developed.

2. Yes - the research shows that algae are more sensitive to complex wastes than other organisms.

3. Yes - the results obtained in the laboratory may differ from the in-situ results since the relationship between the effects of toxicants and the environmental conditions differs in-situ from that in the laboratory. Also the static test using laboratory grown algae may provide different results.

4. Does not apply.

5. The technique may prove useful in setting of effluent limits on a basis of no impact at all.

D. Technique Applicability

1. The technique has a potential to be used as a screening tool across a variety of sites, but more testing should be done.

2. This flow through algal assay performed on portions of indigenous algal communities may be an important source of information as an input to models, especially those dealing with the food chain and the bioaccumulation of toxicants.

3. The technique may be useful in setting site specific water quality guidelines, as it uses portions of indigenous algal populations which are characteristic of the investigated sites.

4. The technique could be used in monitoring of compliance, but more research on the technique itself needs to be conducted. The major drawback is that the tests are time consuming, as the estimation of algal biomass as volume (hence this is an analysis of multispecie community) required the microscope counting technique.

Kaministiquia River Sportfish

Sportfish have been collected from the Kaministiquia River area in 1979, 1981, 1985 and 1987. There have been four sampling locations : near Highway 61, between the Turning Basin and the Mission River, at the river mouth, and at the Mission River mouth (see sampling location map). The principle species collected were walleye, northern pike and white sucker, and a complete listing of the species sampled, number collected and year collected is included in Tables 1 and 2. Only three fish were collected in the 1987 collection, and these results are as yet not available.

The samples were collected by staff of the Ministry of Natural Resources Thunder Bay office and were submitted for analysis as skinless, boneless dorsal fillets to the Ministry of the Environment laboratories in either Toronto or Thunder Bay. The results were interpreted by staff of the Ministry of the Environment Sport Fish Contaminant Monitoring Program and were published in the "Guide to Eating Ontario Sport Fish", which provides anglers and consumers with information on the recommended levels of consumption of sportfish from over 1500 locations in Ontario, and has been published annually since 1978 (MOE/MNR, 1988). The consumption advice in the "Guide" is based on guidelines established by Health and Welfare Canada for allowable levels of contaminants in commercially marketed fish.

Results

a) Mercury

i) Kaministiquia River

Walleye were collected at the Highway 61 site in 1979 and 1981. In 1979, walleye up to 41 cm were suitable for unlimited consumption (ie. < 0.5 ppm), while in 1981, only walleye up to 32 cm were suitable for unlimited consumption. Walleye up to 52 cm collected at the mouth in 1979 were suitable for unlimited consumption.

The mean mercury value for walleye was higher at the Highway 61 site, but the mean size sampled at the mouth was much smaller (Table 1). If, for comparison purposes, the lengths are standardized to 40 cm for the 1981 upstream site walleye (larger sample size in 1981 than in 1979) and for the 1979 walleye at the mouth, the mercury values for that length were higher in the river location than at the mouth (0.58 ppm and 0.30 ppm respectively).

Northern pike collected at the mouth in 1979 were suitable for unlimited consumption up to 64 cm. Since the northern pike is a top predator, the larger pike sampled had accumulated mercury levels which were above the 0.5 ppm guideline, but none of the fish sampled were above the 1.5 ppm guideline for no consumption.

White suckers were collected at two sites in 1979, and the mean mercury values were low and very similar. All samples (up to 45 cm) collected at the Highway 61 site were below the 0.5 ppm guideline for unlimited consumption. White suckers up to 48 cm, taken from the river mouth, were also below 0.5 ppm mercury.

ii) Mission River Mouth

Walleye sampled in 1979 and 1985 were quite similar in mercury content, as the size limit for unlimited consumption was 48 cm and 53 cm respectively, and the mean mercury concentrations were 0.29 ppm and 0.25 ppm respectively in 1979 and 1985. These values are typical of values in other Lake Superior locations.

Northern pike samples were larger in 1985 than in 1979, therefore the mercury concentrations were higher; however, when the samples were standardized to 45 cm to compare the two sets of results, the mercury concentration for both years at that length was 0.17 ppm. Although the northern pike samples did not contain very large fish, all the northern pike within the sampled sizes were suitable for unlimited consumption.

Concentrations of mercury in white suckers were low in both years and all white suckers within the sample sizes were suitable for unlimited consumption.

b) Organics

i) Kaministiquia River

In 1979, analyses for PCB, mirex, heptachlor and heptachlor epoxide was done on all three species at the river mouth, while the walleye at the Highway 61 location were analyzed only for PCB and mirex.

PCB was not detected from the walleye at either site, while the white sucker and northern pike samples both had very low ranges (30 - 380 ppb and not detected - 150 ppb respectively), which were well below the 2000 ppb guideline for unlimited consumption.

Mirex has not been found in samples of Lake Superior fish and is restricted to Lake Ontario, therefore no mirex was detected in any of the Kaministiquia River samples. Heptachlor and heptachlor epoxide, which are normally not detected in Lake Superior fish samples, were not detected in any of the samples from this area.

ii) Mission River Mouth

The 1979 samples were similar to the Kaministiquia River samples in that only PCB, mirex, heptachlor and heptachlor epoxide were analyzed. However, in 1985, a more thorough contaminant analyses was completed, with the following additions to the 1979 list: hexachlorobenzene; aldrin; A-BHC, B-BHC, and G-BHC hexachlorocyclohexane; A- and G- chlordane; toxaphene; octachlorostyrene; pp-DDD; pp-DDE; op- and pp- DDT; and 2,3,7,8-TCDD (Dioxin).

Table 2 shows that the PCB levels were higher in 1985 than in 1979 in the three main species. The concentrations remain at very low levels compared to the 2000 ppb consumption restriction guideline.

Mirex was not detected for any species in either sampling year, and the remainder of the contaminants sampled in the two years were in most cases not detected. The contaminants which were detected were found in very low concentrations.

Discussion

Mercury was found to be the only contaminant to cause restrictions on unlimited consumption of sportfish in the Kaministiquia River and the Mission River. The values for all organics tested for in both rivers were found to be at low or non-detectable levels. The consumption guidelines for the species sampled are contained in the 1988 "Guide to Eating Ontario Sport Fish." The restriction on the unlimited consumption of the larger walleye and northern pike sampled is typical of such predator fish, which are at the top of the food chain and therefore accumulate larger quantities of mercury. The mercury values in the sampled size ranges were typical of those found in other Lake Superior locations.

As a result of the insufficient sample collected in 1987 at the Turning Basin area, there is still a need for a more recent set of results for this section of the Kaministiquia River. An increasing number of contaminants are now being analyzed in sportfish, therefore an updated sampling would give a more thorough indication of the contaminant concentrations in Kaministiquia River sportfish. A collection of sportfish is to be undertaken in 1988 for the area between the Turning Basin and the Mission River. If sufficient samples can be obtained in this collection, then these updated results can be compared to the previous data, and will improve the Kaministiquia River database.

Discussion of Sampling and Analyses

There are limitations in the sampling procedures, as the samples are collected by another ministry - ie. the Ministry of Natural Resources. The staff of the Ministry of the Environment Sport Fish Contaminant Monitoring Program can request a survey of an area and suggest species to be collected, however, it is still necessary for the Ministry of Natural Resources to schedule the time and staff to meet this request. When the sampling does take place, there is always the possibility (if there is a limited amount of time available at the particular location) of such problems as poor weather conditions or insufficient numbers of the species present in the area at that time. Normally, 20 of each species (in a variety of size ranges) are requested to provide sufficient results for establishing consumption guidelines, however, smaller numbers are used if there is a good size distribution in the sample.

Unless specified as a priority (and depending on the sample backlog), a sportfish collection may take a year or longer to be completely analyzed for organics and six months or more for only mercury analyses. The analyzed results are checked by staff at the Labs and by the staff in the Sport Fish Contaminant Monitoring Program. High values are noted and checked against other parameters, and a retest is requested if the value is suspect. Routine analyses of sportfish by the Ministry of the Environment has been an on-going process for a number of years, and approximately 6000 fish are analyzed per year. The results, after the quality checks, are considered to be very reliable.

Sportfish are considered to be good biological indicators of water quality. However, some species may travel significant distances (this is most applicable when sampling species such as salmon and trout from river mouth areas) and therefore to study a potentially localized problem, it is best to sample the resident warm-water species such as northern pike, smallmouth bass, yellow perch, and suckers. The contaminant results for these resident fish populations can then be used along with the results of samples of young-of-the-year fish, such as yellow perch and spottailed shiners, to give a good indication of localized contaminant conditions.

Table 1. Mercury and PCB Results for 1979 and 1981 Kaministiquia River Sportfish Samples

Highway 61 Site

1979

Species	#	Length (cm) Mean	Length (cm) Range	Mercury (ppm) Mean	Mercury (ppm) Range
Walleye	6	43.7	33.5-52.0	0.70	0.31-1.46
Northern Pike	1	68.0	-	0.41	-
White Sucker	9	35.0	30.5-43.5	0.24	0.10-0.39

Species	#	Length (cm) Mean	Length (cm) Range	PCB (ppb) Mean	PCB (ppb) Range
Walleye	5	45.7	38.0-52.0	nd	nd

1981

Species	#	Length (cm) Mean	Length (cm) Range	Mercury (ppm) Mean	Mercury (ppm) Range
Walleye	13	48.6	23.5-61.7	0.68	0.41-1.20

River Mouth 1979

Species	#	Length (cm) Mean	Length (cm) Range	Mercury (ppm) Mean	Mercury (ppm) Range
Walleye	14	34.0	21.0-49.5	0.23	0.10-0.73
Northern Pike	15	62.2	44.5-82.0	0.52	0.21-1.17
White Sucker	15	36.7	21.0-47.0	0.28	0.07-0.80

Species	#	Length (cm) Mean	Length (cm) Range	PCB (ppb) Mean	PCB (ppb) Range
Walleye	5	39.7	36.0-49.5	nd	nd
Northern Pike	15	62.2	44.5-82.0	43	nd-150
White Sucker	15	36.7	21.0-47.0	154	30-380

Turning Basin to Mission River 1987

Species	#	Length (cm) Mean	Length (cm) Range	
Northern Pike	1	46.3	-	results not yet available
Longnose Sucker	2	40.6	33.9-47.2	

nd = not detected

Table 2. Mercury and PCB Results for 1979 and 1985 Mission River Mouth Sportfish Samples

1979

		Length (cm)		Mercury (ppm)	
Species	#	Mean	Range	Mean	Range
Walleye	8	39.8	31.0-45.5	0.29	0.11-0.45
Northern Pike	10	40.5	27.5-50.5	0.16	0.10-0.24
White Sucker	15	32.7	22.0-41.0	0.13	0.07-0.25

				PCB (ppb)	
				Mean	Range
Walleye	5	42.3	22.0-41.0	nd	nd
Northern Pike	5	47.2	40.5-50.5	20	nd-60
White Sucker	15	32.7	22.0-41.0	70	nd-290

1985

		Length (cm)		Mercury (ppm)	
Species	#	Mean	Range	Mean	Range
Walleye	29	38.0	28.0-58.2	0.25	0.12-0.80
Northern Pike	9	53.4	32.8-72.0	0.26	0.08-0.47
White Sucker	20	42.5	35.2-48.0	0.18	0.07-0.36
Lnose Sucker	20	39.3	28.2-45.3	0.26	0.03-0.90
Yellow Perch	2	27.9	19.4-36.4	0.39	0.14-0.64

				PCB (ppb)	
				Mean	Range
Walleye	29	38.0	28.0-58.2	64	nd-424
Northern Pike	9	53.4	32.8-72.0	37	nd-92
White Sucker	20	42.5	35.2-48.0	154	nd-350
Lnose Sucker	20	39.3	28.2-45.3	437	40-2070
Yellow Perch	2	27.9	19.4-36.4	36	21-50

nd= not detected
Lnose Sucker = Longnose Sucker

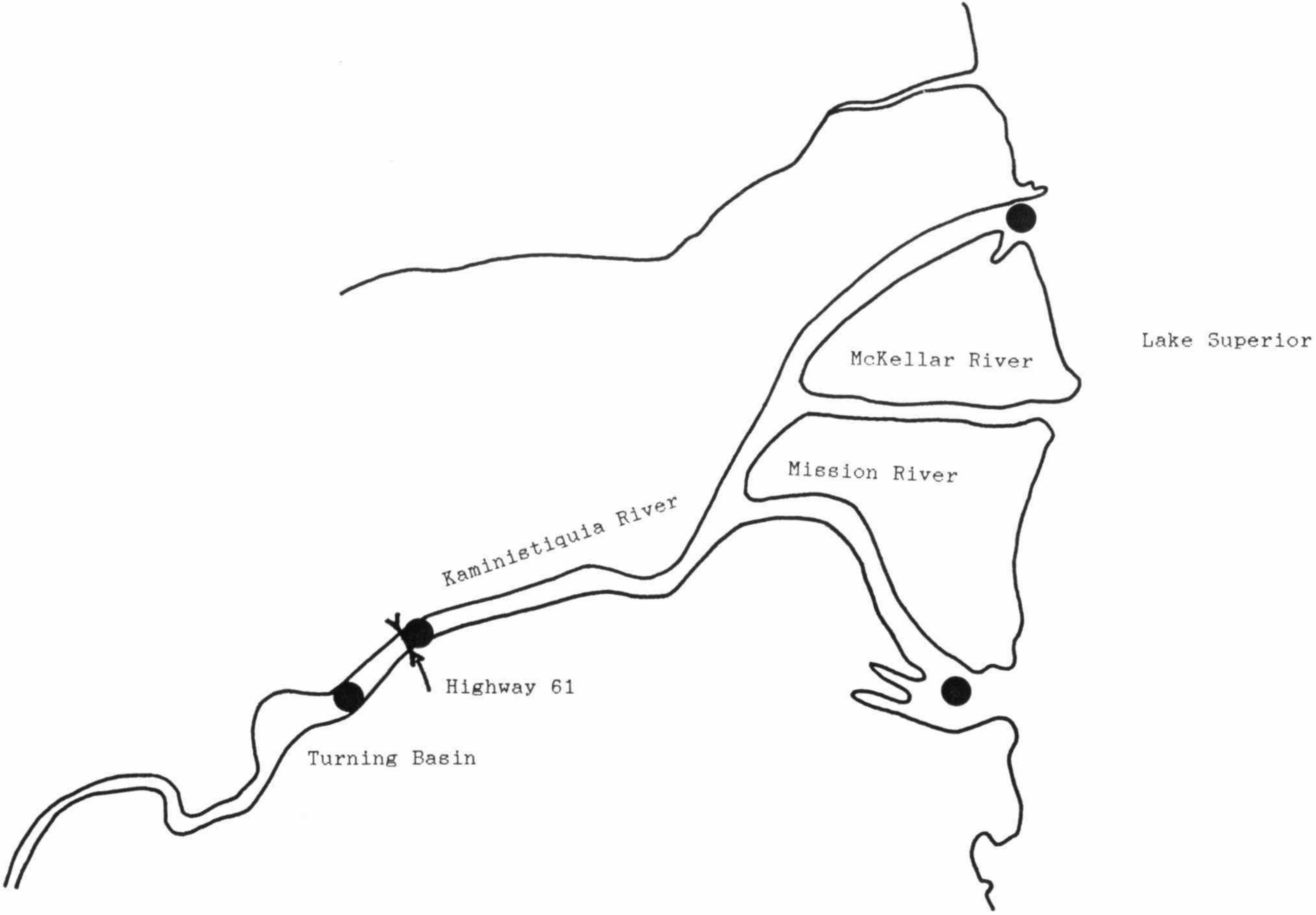


Figure 1. Sampling Sites for Kaministiquia River Sportfish Collections

INTRODUCTION

Indigenous young-of-the-year yellow perch were used as biomonitors to address the primary objective of the Kaministiquia River MISA Pilot Site Study: the effect of Canadian Pacific Forest Product effluent on contaminant availability in the river. Spottail shiner residue data are also presented for a seven year interval (1979-1986) to assess temporal trends and document baseline conditions for contaminant availability over an extended time interval.

METHODS

Young-of-the-year yellow perch were collected in 1985 above and below the Canadian Pacific Forest Products plant discharge, and young-of-the-year spottail shiners have been collected over a seven year interval (1979-1986) at the Kaministiquia River estuary (Figure 1). Individual fish were measured (T.L.) and wrapped as ten fish composites in hexane-rinsed aluminum foil. Samples were stored at -20°C for about three to four months before analyses.

Contaminant residues were identified and quantified at the Ontario Ministry of the Environment Laboratory in Rexdale. Packed column gas chromatography was used for PCB, HCB, heptachlor, mirex, aldrin, octachlorostyrene and p,p'DDE analyses. Capillary gas chromatography was used for chlordane, BHC, o,p'DDT and p,p'DDT residues. Simultaneous, dual capillary methods were used for chlorobenzene and chlorophenol residue determination. Total mercury residues were determined by standard cold vapour flameless atomic absorption.

RESULTS AND DISCUSSION

Spatial Distribution

Although chlorinated phenol residues have been detected in the effluent of Canadian Pacific Forest Products, and they accumulate in caged fish (K. Flood pers. comm.), no residues were found in yellow perch collections 3 km downstream from the plant discharge or in spottail shiners from the Kaministiquia River estuary (Table 1, Table 2).

Low concentrations of hexachlorobenzene, PCBs and mercury were found in the 1985 perch collections from the upper and lower parts of the river. PCB accumulations were significantly ($p < 0.01$) higher in perch collections from the industrialized, lower part of the river, whereas mercury residues were higher in collections from the upper river. While PCB availability downstream of the Canadian Pacific Forest Products was clearly elevated, results from this survey did not identify PCB sources, nor is it known why mercury was more available in the upper part of the river. None of the contaminant levels in the forage fish exceeded the IJC Aquatic Life Guidelines.

Heptachlor, aldrin, mirex, DDT, BHC, chlordane, octachlorostyrene, hexachloroethane, hexachlorobutadiene, and trichlorotoluene were not detected in any of the fish collections from the river.

Temporal Trends

No trends were evident for any of the contaminants analyzed from the limited data base available.

Figure I. Kam River juvenile fish collection sites.

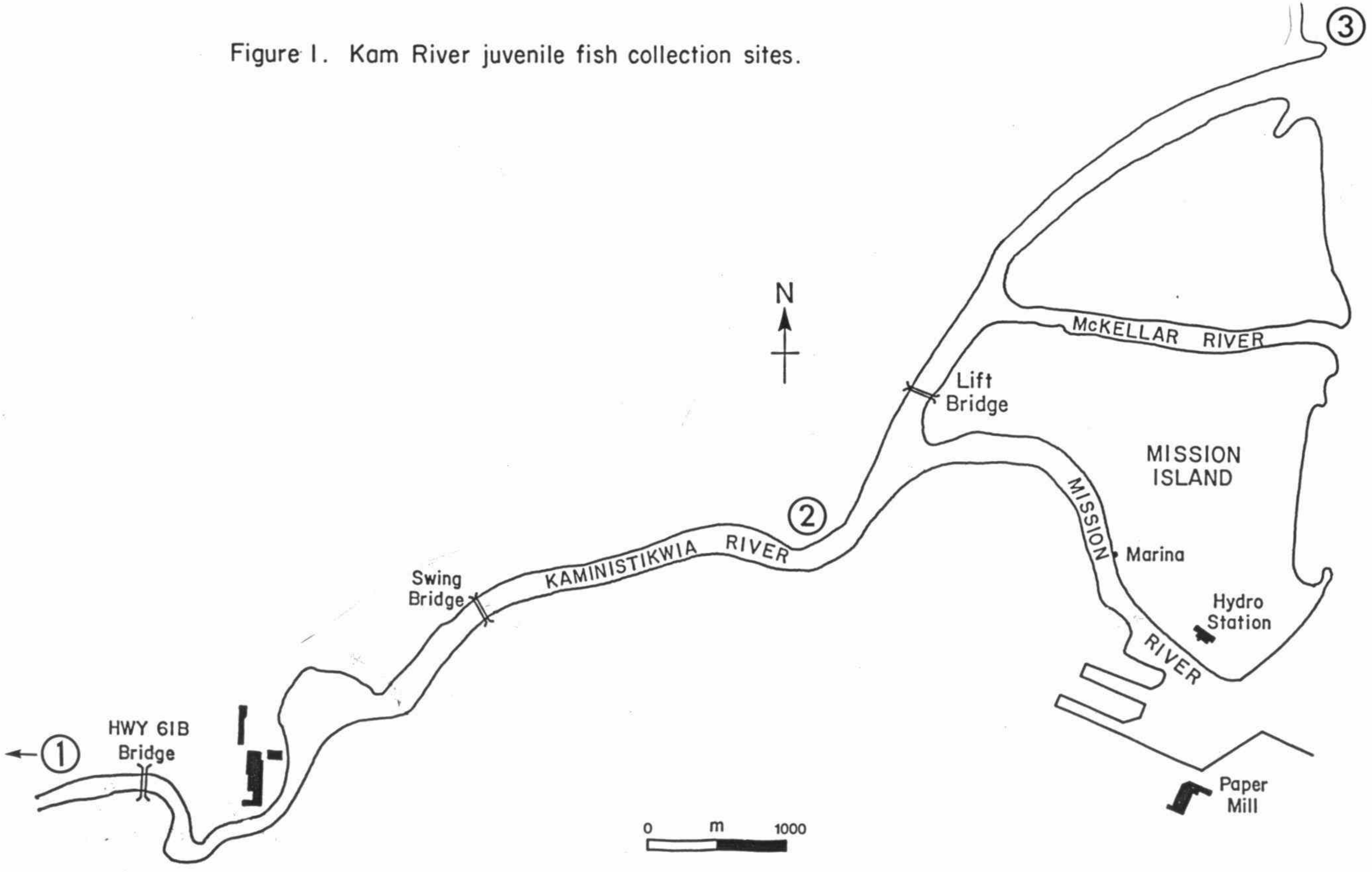


Table 1: Organochlorine and mercury residues in young-of-the-year yellow perch from the Kaministiquia River - 1985. (values shown are means ± SD wet weight)

Site No.	Collection Site	Year	N	Fish Size (mm)	% Fat	Tri, Tetra Penta, Chlorophenols	Tri, Tetra, Penta, Chlorobenzene	HCB	PCB	Mercury
1.	Control about 5 km above Canadian Pacific Forest Products Plant	1985	7	65 ± 3	2.6 ± .3	ND	ND	4 ± 2	20 ± 14	84 ± 15
2.	About 3 km below Canadian Pacific Forest Products Plant near Mission River confluence.	1985	5	62 ± 3	2.7 ± .1	ND	ND	6 ± 1	84 ± 17	28 ± 4
	DETECTION LIMITS					50	2	1	20	10

ND - Not detected

IJC Aquatic Life Guideline	100	500

Table 2: Organochlorine residues in young-of-the-year spottail shiners from the Kaministiquia River estuary. (values shown are means ± SD wet weight).

Site No.	Year	N	Fish Size (mm)	% Fat	Tri, Tetra, Penta Chlorophenols	Tri, Tetra, Penta Chlorobenzene	HCB	PCB
3	1979	8	30 ± 4	2.0 ± .6	NA	NA	ND	21 ± 14
3	1983	7	37 ± 3	3.2 ± .7	ND	ND	ND	82 ± 13
3	1984	7	37 ± 3	1.4 ± .3	NA	NA	ND	64 ± 10
3	1986	4	30 ± 3	1.0 ± .3	ND	ND	ND	ND
DETECTION LIMITS					50	2	1	20

NA - Not available
ND - Not detected

MISA PILOT SITE ASSESSMENT TECHNIQUE QUESTIONNAIRE
JUVENILE FORAGE FISH

A. Field Work and Laboratory Analyses

1. Question too vague. Needs defining as to what are the objectives of the survey i.e. single point source, or impact zone assessment etc.

2. Seine net, boat, two person team with some basic training. Analyses can be done at outside laboratories.

3. Habitat suitability is critical for sample collection. Field work restricted to a 6-month period (May-October).

4. Turnabout time dependant on analytical support. Data quantity related to survey objectives.

5. None.

6. Technique can be mastered with minimal training.

B. Interpretation of Results

1. Question too vague. Needs to be more specific i.e. type of assessment, level of interpretation.

2. None.

3. Detection limits related to contaminant type. Little need for confirming studies since technique offers results that integrate chemical and biological variability over time.

4. Technique does not have enough resolution to assess impact from a specific point source.

5. Results are compound-specific.

C. Technique Utility within MISA Context

1. Some internationally recognized guidelines and criteria exist for several compounds. Further criteria need to be developed.

2. No.

3. No.

4. Question not specific enough.

5. i) None
   ii) None
   iii) None
   iv) None
   v) Useful in baseline assessment, evaluation of control measures and on-going monitoring, provided that above objectives have wider application than specific point-source assessment.

D. Technique Applicability

1. Not in the context of narrow MISA objectives related to a specific point-source.

2. No.

3. No.

4. No.

KAM RIVER STUDY - MICROBIOLOGY COMPONENT

OBJECTIVES

1. Determine the contribution of Great Lakes Forest Products (GLFP) pulp and paper mill and other industrial and municipal effluents on the bacteriological quality of the Kam River.
2. Provide comparative data on the occurrence and magnitude of various Klebsiella species in pulp and paper mill effluent.
3. Determine the overall bacteriological loading in the Kam River system.
4. Aid in defining the impact of the Kam River system on the water quality of the Thunder Bay Harbour nearshore.

SAMPLING STRATEGY

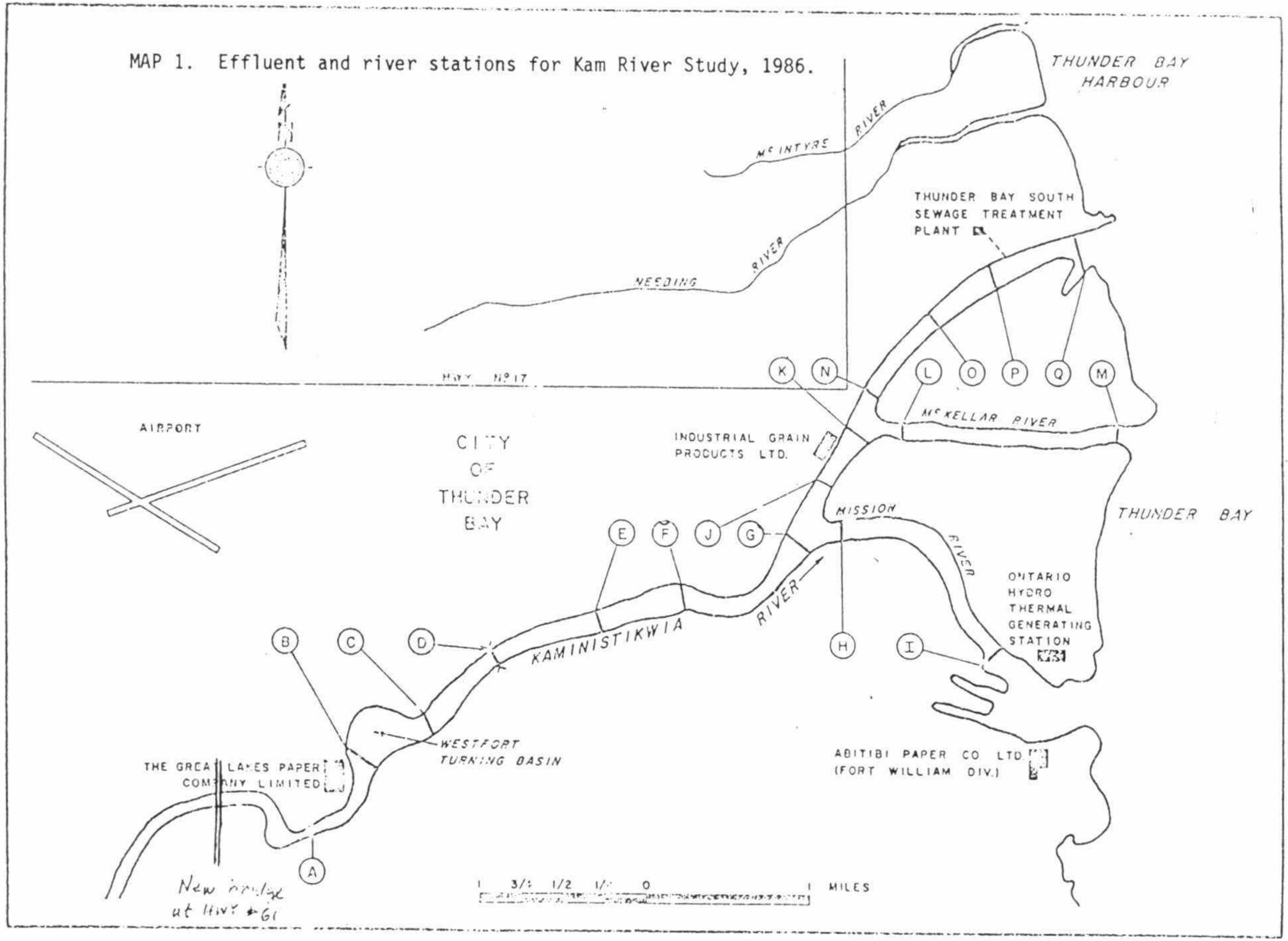
SAMPLING LOCATIONS

Effluent Stations

GLA	GLFP - Main mill effluent
GLB	GLFP - Clean water effluent
IGP	International Grain Products - Final effluent
STP	Thunder Bay WPCP - Final effluent

River Stations (See Map 1)

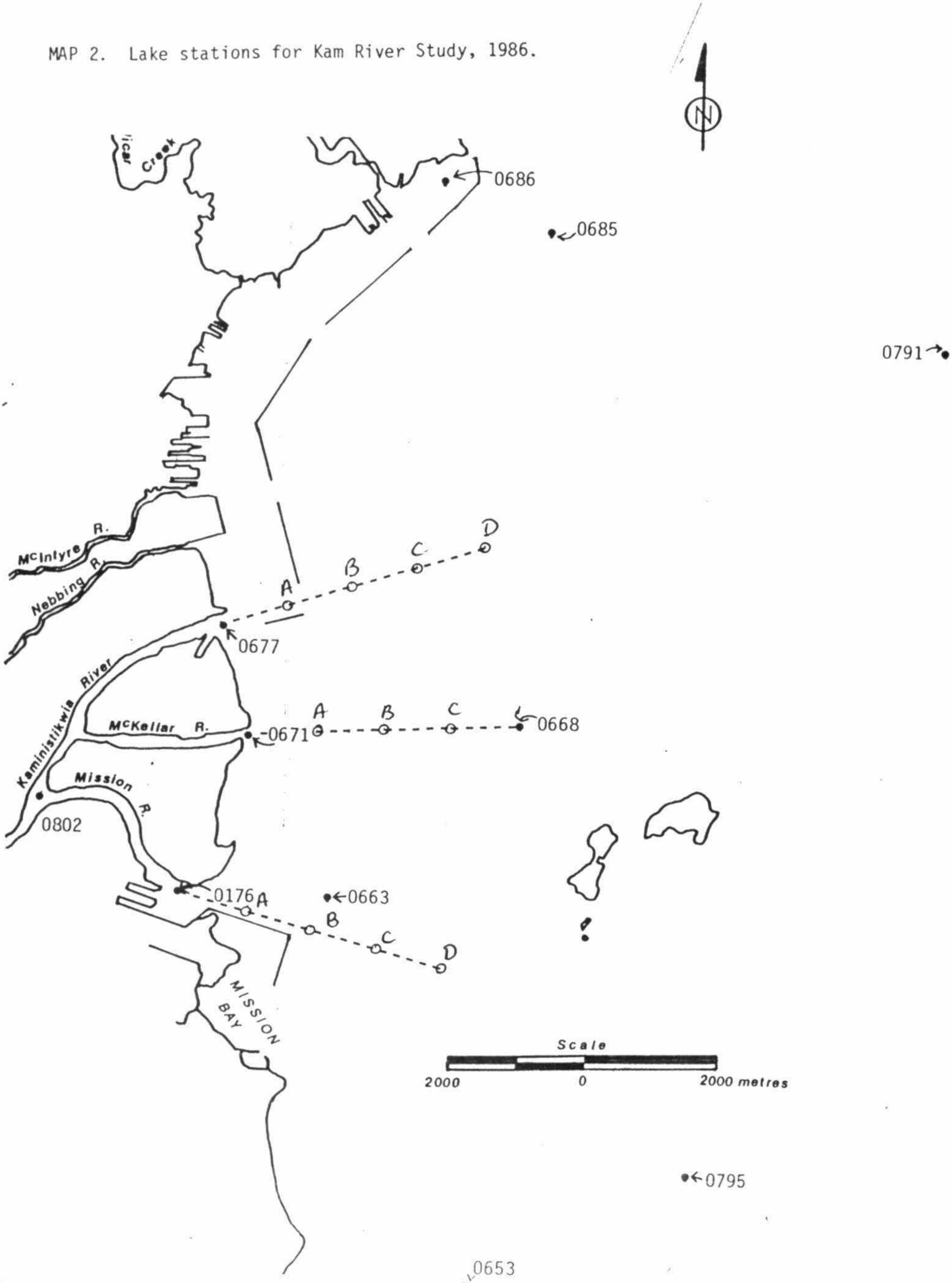
A	Kam R. - Upstream GLFP
BL	Kam R. - Below GLFP - Left side of river
BM	Kam R. - Below GLFP - Midstream
BR	Kam R. - Below GLFP - Right side of river
D	Kam R. - At bridge, Highway 61B
G	Kam R. - Above junction of Mission River
H	Mission R. - Below junction of Kam River
I	Mission R. - Mouth
L	Kam R. - Above IGP
KL	Kam R. - Below IGP - Left side of river
KM	Kam R. - Below IGP - Midstream
KR	Kam R. - Below IGP - Right side of river
L	McKellar R. - Below junction of Kam River
M	McKellar R. - Mouth
PM	Kam R. - Above T. Bay STP - Midstream
Q	Kam R. - Mouth



MAP 1. Effluent and river stations for Kam River Study, 1986.

Fig. 1 Sampling ranges (A-Q) - Lower Kaministikwia River

MAP 2. Lake stations for Kam River Study, 1986.



Lake Stations (See Map 2)

677	Kam R. mouth
677A	Lake Superior, out from Kam R. mouth
677B	" " " " " " "
677C	" " " " " " "
677D	" " " " " " "
671	McKellar R. mouth
671A	Lake Superior, out from McKellar R. mouth
671B	" " " " " " "
671C	" " " " " " "
668	" " " " " " "
176	Mission R. mouth
176A	Lake Superior, out from Mission R. mouth
176B	" " " " " " "
176C	" " " " " " "
176D	" " " " " " "
0795	Lake Superior, Control station
0791	Lake Superior, Control station

SAMPLING FREQUENCY

Effluent Stations

The effluent stations were sampled approximately every 8 hours starting at 20:00 hours August 11, 1986 and continuing until 08:00 hours, August 14, 1986.

River Stations

The river stations were sampled once per day at approximately 08:00 hours for three consecutive days. The river stations were sampled August 12, 13, and 14, 1986.

Lake Stations

The lake stations were sampled once per day on August 14, 15, and 16, 1986.

BACTERIOLOGICAL PARAMETERS

Effluent Stations

Samples from the effluent stations were analyzed for total coliform bacteria (TC), background bacteria on the total coliform plate (BKD), *E. coli* (EC) and *Klebsiella* species (KP).

River Stations

Samples from the rivers stations were analyzed for the same bacteriological parameters as the effluent samples.

Lake Stations

Samples from the lake stations were analyzed for heterotrophic bacteria (HB20) in addition TC, BKD and KP. These samples were not analyzed for EC.

BACTERIOLOGICAL METHODS

TC and BKD were analyzed by membrane filtration (MF) using m-endo agar LES at 35 C for 22 hours. KP was analyzed by MF using m-Kleb agar at 35 C for 24 hours. EC was analyzed by most probable number (MPN) method using LTB-MUG broth at 35 C. HB20 was analyzed by spot plate method using CPS agar at 20 C for 7 days. Some Klebsiella colonies were further identified using API20E biochemical identification kits.

RESULTS AND DISCUSSION

1. EFFLUENTS

GLFP Effluent

Two effluent streams from Great Lakes Forest Products were monitored: the main mill effluent (station # GLA) and the clean water effluent (station # GLB). The main mill effluent contained extremely high bacterial levels while the clean water effluent was virtually free of indicator bacteria.

The geometric mean TC level (GM-TC) in the main mill effluent was 10,456,000 per 100 ml. (Table 1.) This value was not considered unusual as the total coliform group of bacteria is commonly found at high levels in pulp and paper mill effluents. During the period of this survey, the level of total coliform bacteria in this effluent was relatively constant, ranging from a low of 4,000,000 to a high of 27,000,000.

Almost all of the total coliform component in the main mill effluent was made up of Klebsiella bacteria. These organisms were present at a geometric mean level (GM-KP) of 9,749,000. The predominant Klebsiella species in this effluent was K. pneumoniae.

The geometric mean EC level (GM-EC) in the main mill effluent was greater than 1400. The exact level was unknown as several of the samples exceeded the upper limit of the testing range used in the laboratory. The level of EC in this effluent was higher than expected, given that it apparently should contain no domestic wastes. Interference in the EC test method by Klebsiella was discounted by confirming the presence of E.coli in the MPN tubes.

TABLE 1. Geometric means per 100 ml for bacterial parameters from effluent and river stations, August 12-14, 1986.

STATION	BACTERIAL PARAMETERS				
#	TC	BKD	EC	KP	HB
Effluent Stations					
GLA	10,456,000	51,043,000	G1400	9,749,000	n/a
GLB	22	46	L10	L10	n/a
IGP	37,000	1,498,000	167	43,000	n/a
STP	n/a	n/a	n/a	n/a	n/a
River Stations - Kam River					
A	769	6,044	21	L10	n/a
BL	837,000	1,568,000	371	525,000	n/a
BM	384,000	2,143,000	158	968,000	n/a
BR	949,000	5,880,000	136	894,000	n/a
D	507,000	1,142,000	136	726,000	n/a
G	207,000	409,000	53	144,000	n/a
J	155,000	120,000	92	93,000	n/a
KL	100,000	67,900	200	98,000	n/a
KM	102,000	156,000	76	79,000	n/a
KR	165,000	104,000	93	109,000	n/a
PM	31,000	37,000	354	17,000	n/a
Q	48,000	98,000	147	37,000	n/a
River Stations - McKellar River					
L	62,000	143,000	197	70,000	n/a
M	22,000	29,000	54	16,000	n/a
River Stations - Mission River					
H	169,000	237,000	45	148,000	n/a
I	68,000	49,000	24	85,000	n/a

G = Greater than value shown L = Less than value shown

IGP Effluent

Samples collected from the effluent at IGP between 19:50 on August 11 and 19:50 on August 12 contained less than 1000 TC, high levels of BKD that ranged from 120,000 to 40,0000,000, minor levels of EC (less than 3 to 240), and less than 1000 KP per 100 ml. A more precise quantification of TC and KP was not possible due to the interference of the high BKD levels and suspended material in the samples. These results are apparently typical of the normal operating conditions of this plant.

However, the samples collected between 04:00 and 19:55 on August 13, showed a distinctly different set of conditions. These samples were white in colour (compared to the normal brown) and contained elevated levels of indicator bacteria. The TC levels increased to 13,000,000, EC increased to greater than 2400 and KP to greater than 10,000,000. Apparently, during this time period, IGP had a break in one of their pipes and their wastes were being dumped virtually untreated into the Kam River. Once this break was repaired, the colour of the effluent was brown again and the bacterial levels returned to normal.

The geometric means for TC, EC and KP are misleading and should be disregarded. These high values are a result of an abnormal occurrence and do not represent the normal values for the effluent for this plant.

Thunder Bay WPCP Effluent

Unfortunately, the majority of samples collected from the Thunder Bay WPCP were sampled incorrectly. These samples were collected without making allowance for the proper chlorine contact time. Consequently, the bacteriological results were extremely high and cannot be used. Only the final sample in the series was sampled correctly. This sample had a TC count of 56,000, an EC count of 570 and a KP count of 1,100. These latter results are typical of those obtained during the regular monthly monitoring of this effluent.

2. OVERALL BACTERIOLOGICAL LOADING OF KAM RIVER SYSTEM

Samples collected from the Kam River above Great Lakes Forest Products (GLFP) at station # A (Map 1) contained relatively low levels of bacteria (Table 1). The geometric mean for total coliform bacteria (GM-TC) at station # A was 769 per 100 ml. The geometric means for E. coli (GM-EC) and Klebsiella (GM-KP) were 21 and 10 per 100 ml, respectively. The KP level was at the lower limit of detection of the method used for this parameter.

TABLE 2. Geometric means per 100 ml for bacterial parameters from river mouths and lake stations, August 14-16, 1986.

STATION	BACTERIAL PARAMETERS				
#	TC	BKD	EC	KP	HB
Lake Stations - out from Kam River					
0677	13,000	18,000	n/a	6,400	905,000
0677A	1,900	16,000	n/a	630	286,000
0677B	530	940	n/a	140	21,500
0677C	650	1250	n/a	450	11,000
0677D	74	235	n/a	52	2,800
Lake Stations - out from McKellar River					
0671	14,000	29,900	n/a	4,000	1,067,000
0671A	400	680	n/a	290	43,000
0671B	450	1,000	n/a	280	20,000
0671C	200	540	n/a	110	12,000
0668	240	260	n/a	70	10,000
Lake Stations - out from Mission River					
0176	64,000	106,000	n/a	39,000	G464,000
0176A	32,000	34,000	n/a	18,000	G802,000
0176B	14,000	20,000	n/a	8,400	G100,000
0176C	106	277	n/a	59	13,000
0176D	59	237	n/a	48	5,800
Lake Stations - Control Stations					
0791	60	42	n/a	17	6,800
0795	40	88	n/a	10	12,400

G = Greater than value shown L = Less than value shown

The bacterial levels observed at this station were relatively low, arising from a combination of agricultural, municipal and private sources upstream. These bacterial levels were consistent with those observed via samples from other sampling programs and shall be considered the baseline loading for this river system above the influence of major industrial or municipal sources.

At the three stations just below GLFP, the bacterial levels were dramatically higher. GM-TC levels were 837,000, 384,000 and 949,000 at stations BL,BM and BR, respectively. These levels had increased 1000 fold over the baseline loading. There was a even larger (100,000 fold) increase in KP levels. The greater increase for KP was due to the fact that there was little or no KP in the water upstream of GLFP while the effluent from GLFP was almost entirely composed of KP.

GM-EC levels increased ten fold to 371, 158 and 136. As EC is a fecal coliform, water containing these levels of fecal coliforms would be considered unsuitable for bathing.

Downstream at bridge at Hwy 61B (station # D), the levels of all the bacterial parameters fell. Further downstream at station # G (above junction of Mission R.), and station # J (above IGP), the bacterial levels continued to fall. At these latter two locations, the GM-TC level decreased to 206,000 and 154,000, respectively. The GM-EC level decreased to 53 and 92, while the GM-KP level decreased to 144,000 and 93,000.

At station # K (below IGP), the geometric means for the bacterial parameters continued to fall. There was no discernible impact on the bacteriological water quality of the Kam River as a result of the effluent from IGP. This was probably a combination of the infrequent sampling (samples from the river were collected only on a daily basis), and the already high bacterial loading of the river at this point.

The bacteriological levels at the next station (PM) downstream on the Kam were somewhat ambivalent. The GM-TC and GM-KP levels both declined slightly (31,000 and 17,000), while the GM-EC level increased (354). It is probable that this increase was not a real trend but simply a result of fluctuations in the limited data at the river stations. It was observed that there were substantial fluctuations in the bacteriological data for stations located in a transect across the river. There are no major effluents between stations # K and PM that would account for the increased level of EC.

The trend in bacteriological levels at station # Q (below STP) was again uncertain. GM-TC and GM-KP levels were slightly higher, but the GM-EC level fell. The effluent from the Thunder Bay WPCP may have had some impact on the bacteriological water quality, but again, the limited data and the normal fluctuations in the data preclude making any definitive conclusions. Nevertheless, the bacterial levels at the mouth of the Kam River were elevated above the baseline by 100 fold for TC, 10 fold for EC, and 10,000 fold for KP. A similar pattern was observed at the mouths of the McKellar and Mission.

3. IMPACT OF KAM RIVER SYSTEM ON THUNDER BAY NEARSHORE

Bacterial samples were collected at 2 control station in Lake Superior: # 0791 and 0795 (Map 2). These stations were located beyond the expected influence of the Kam River system. However, the data at these two stations were above typical baseline values observed during other surveys of Lake Superior (Table 2). Nevertheless, these stations will be used as baseline for comparison to the transect data out from the river mouths.

As expected, as the distance from the Kam River system increased, the bacterial levels of all parameters decreased. However, even the last stations in each transect had bacterial levels somewhat above the control stations.

CONCLUSIONS

1. The effluent from GLFP was the major contributor to the high indicator bacterial levels in the Kam River system. This effluent could be traced to the river mouths and out into Lake Superior almost to the Welcome Islands using TC and KP bacteria.

2. High levels of Klebsiella pneumoniae were found in the main effluent from GLFP. These bacteria were present normally only in relatively low numbers in the other major effluents going into the Kam River.

3. Although the effluent did not contain particularly high levels of E. coli (compared to effluents from some sewage treatment plants), due to the volume of flow, this effluent must be considered the prime source of EC in the Kam River system.

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Kaministiquia river MISA pilot
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